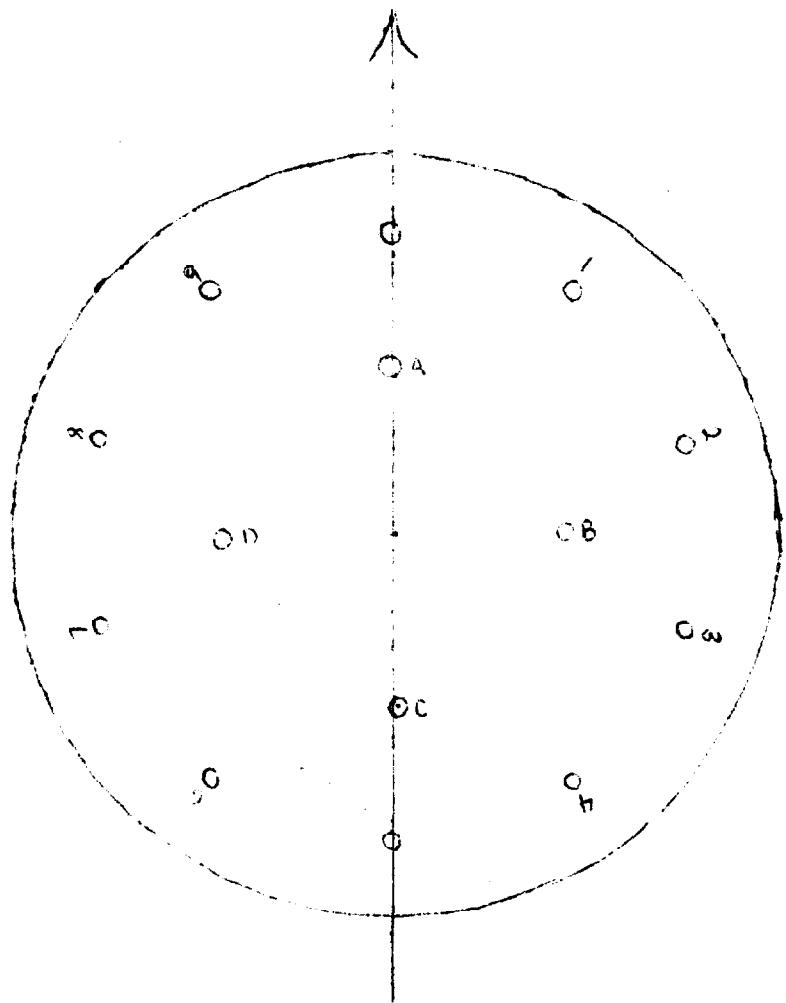


YALE UNIVERSITY
OSBORN BOTANICAL LABORATORY
NEW HAVEN, CONNECTICUT

EXPERIMENTS IN THE GENETICS OF BACTERIA

1946- 1947.

Joshua Lederberg.



AUXANOGRAM STENCIL -

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Abbreviations:

F(x) = Fries supplemented \bar{x} . (*Mucoropora minimal*).
CM = complete medium (essentially aminoac + vits.)

Ca = HC = Hydrolyzed casein.

Vit = Vitameric B + hydrolyzed YNA

YNA = yeast nucleic acid

EAA = EA "essential" amino acids :

NEAA = NA non - EAA.

O = minimal

∞ = complete

T(x) = coll O = Tatum + Gray's + mid. supal.

Amino acids.

- | | | |
|---|---------------------|---------------|
| 1 | leucine | 9. methionine |
| 2 | isoleuc | 10 histidine |
| 3 | valine | 11 threonine |
| 4 | phen. | 12 aspart |
| 5 | trypt. | 13 glutam |
| 6 | tyrosine | 14 proline |
| 7 | lysine | 15 HO- |
| 8 | arginine | 16 glycine |

17 alanine

18 cysteine

6 tyrosine

Ind = indole

Dith = dithiothreitol.

- Vits. V
- | | | |
|---|------------|------------|
| 1 | Thiamin | 8 choline |
| 2 | Riboflavin | 9 inositol |
| 3 | PAB | 10 Biotin |
| 4 | NIC | |
| 5 | folic acid | |
| 6 | puidoxin | |
| 7 | plant | |

Purines + Pyr.

3/23/46. - 3/24

Y 160.

Test 156 - Strains. Dil. surface growth in H₂O + test as indic.

Nov 9P23. 33°

1st Reading: 12 h.

2nd 24 h.

9A24.

#	O	C-M	HC	Vit	Eaa	Naa
1	-	-	++ ++	++ ++	-	-
3	-	-	++ ++	++ ++	-	-
4	-	-	++ ++	++ ++	-	-
7	+	+	++ ++	++ ++	+	+
9	+	++	++ ++	++ ++	-	+
10.	+	+	++ ++	++ ++	+	++

(This agrees w/ the previous interpretation that 7-10 may readily back-mutate. 1, 3, 4 should be tested further. of (1).)

Nov: dil. from HC

9P24. 36 h.

Note poor growth in e.g. g. Evidently, strong inhibition is present. Wh. is inhibitory??

1: T(1)

hypot -

sol. - ++

thr -

lys -

arg -

dal -

meth -

v-i ~~+~~ ++

luc - +

hist -

HC ++

O -

Repeat 9: Nov dil from CM. 24 h. 1st. 36 h 2

T(Vit) - +

T(O) ++

T(HC) ++

Vit - +

O ++

Vit + ++

O ++

Test 160 [156-1] for adaptation.

161.

9P26.

37°.

162?

bmoz. into o.

12h. 24h.

~~1 HC~~

o

-

~~2 V-iiso.~~

o

-

~~3 LC~~

o

-

~~4 cool.~~

o

-

Therefore these responses are by way
of growth, rather than adaptation!

1130A18

1130A18

~3.30

~3.30

~~156-9.5 from HC~~

~~" V-iT~~

~~" o.~~

~~onto CM plants for testing~~

++

++

-

+

-

-

~~dilute + test~~

~~2P27.~~

5

6

7

±

±

-

+

-

+

+

Test 160 for growth vs. adaptation - 9P26. into T(0)

12h. 24h. 48h. 930A29 10A30 ~~test~~ 10A29 - 10P29 10A30

1. from HC

-

-

-

-

++

-

±

++

2 " V-i

-

-

-

2P27

3 LC

-

-

-

4 cool

-

-

-

-

-

-

Test 156-9 substrains.

a. to CM plants

5

from HC

6 from vit

7 from Oxyg 4P29 10A30

1130A18 330P18 8P28 A29 Test 3 0

V

to vit + 2P27 HC

{

5-vit

-

+

++

±

#

++

++

++

±

+++

-

+

++

min.

V

{

6-vit

-

+

++

±

#

++

++

++

++

++

This behavior is

remarkable. What is

inhibitory? What is

the type of genetic

modification?

See 164.

Plant identification.
"Aeranography"

163

Jan 7978

histidine

→ c. Hydrolyzed Cescin →

E-8

161-6

ca. 10^{4-5} colonies per plate. They are visible for a radius of 1 cm in both cases around the HC, thus thin out somewhat.
161-6 - not scattered large colonies, but quite numerous small. : not "adaptation": . . . vitamin effect is directly on growth, not adaptation.
Sp. of a.a. not clear. Envelop faster on e.a.a. than on s.c.a.a.
but this may not be a specific response. Need L-15 for control. What by bacteria??
P 37 ca. 10-12 "colonies" full size are seen
in the E-8 plate, presumably adaptants
Try *Acetobacter* *minutum*.

See Aeranography - p. 168.

3/28

Author identification's preliminary.

163a

plate motilinase (E-8) and 161-6 heavily into 1% agar.
When solidified, add a loopful of

- (a) HC
(b) histidine .1% to each.

7P28

Take "washed agar #1" for biotin. Agar washed by 10 transfers through 10% H_2O_2 , + 2x in 95% alcohol, dried in desiccator to 32°C .

Plate "58" (biotinless *E. coli*) in 2% agar unwarmed

P31 - no colonies! "was not + 1 or better"

buc. on surface (steaks) 8 P.M.

A2 - Well developed colonies only where bromelin was added. None elsewhere ! !!

Auto-avow

Test various T-L. mutants.

Re-test 156-1 142-17.

164

Test 156-2, 3.

1130 P 28 mos.
930 A 29.1
12 N

3 98 29

	O	TL	TL(HC)	+ eaa	TL+eaa		
✓ 1	142-17	-- -	-- -	+ + + + - + +	-- -	= 410	
✓ 2	- 36	-- =	- - ^{10A30} ++	+ + + + + + +	-- ++	(adapted?)	
✓ 3	- 54	-- -	- - -	- + + - + +	-- -	= 19	
✓ 4	- 57	-- -	- + + + + + + +	+ + + - + + +	- + + + n. g.		
5							
✓ 6	O	HC	neaa	leuc ^①	isoleuc ^②	val + isoleuc ²³	val + leuc ¹³
7	156-1	-- =	++ + +	-- =	+ + + + - + +		- + + + ± + + + +
8							
9	O	HC	neaa	eaa		check!	
10	156-3	-- -	++ + +	-- =	++ + +		
11							
12	156-4.	-- -	++ + +	-- =	++ + +		
13							
✓ 14	E-1	O ^{10A30}	HC	neaa	eaa ^{10A30}		
15		-- -	++ + + +	+ + +	-- -		
16							
✓ 17	Test 156-9	eaa	O	HC	Vits. V1 V2 V3 V4		
18	various vitamins	++ + +	--	- + + +	-- + + + - + + + - + + +		
19							
* 20	neaa	/ 8 /	V6	V7	V8	V9	V10. VII-yah.
* 21		- + + +	/ 11 /	- + + +	- + + +	- + + +	- - + - + + +
✓ 22	E-6	O → - - -			✓ P3		
✓ 23	E-1 + E-6 (sup).	O →	-- -				
24				10A30			

mos. 530P4. dupl. ^A 1130P Re-test ~~to~~ 142-17- Densograms.

9A 10 - D + + A +. dupl. in vitamins.

Many adapted colonies. 12M10. Very response. -
to new state H P10. bicarbonate 4L before dupl. vits. etc.

12M10 - Thiamin.

antiseptics
enzymes
nutrid

3/29 - Identifying mutants on hand

1. 12N30
2. 4P30

165

12M30 Rec.

12M31 Rec.

1) 4P30 2) 10A1. 3/20

		val	φ	try	lys	arg	meth	cyst
	TL	TL+3	TL-4	TL-5	TL-7	TL-8	TL-9	TL-10
1	142-17					-±	±±++	
2	142-36 N.G.	-++	+++	-++	±++	-++	-++	-++
3	142-54	--	--	--	--	--	-++	--
4	Postpone							
5	more.							
6								
7								
8								
9								

Checks 4/2/46 TL-9 ++ no others.
✓ 4/3/ . ∴ probably both methionine.

0 cont.	11	10	1	2	3	4	5	6	7	8	9
4	156-1			±±*	✓	±	-				
5	156-3	-	--	++	±±++	-	✓	-	✓	-	✓
6	156-4. ±±	--	±++	±±+	-	-	-	-	-	-	-

No further growth by 10A1.

(1) 10A30.

Checks: 4/2 - OK.

* my own
pups.

other is
Tatum's.
may not
be enough
vol.

See info for recheck on 142-17 + 54.

6P4. - Plate 142-17 + 142-54 into 1(TL) agar. Use more:
incubate to 9P4, then do autoradiogram on essential a.g. on 54,
only "9" & 17 using double depth agar.

H2	B	1	2	3	4	5	6	7	8	9	10	C	D	1	Check.
17	++	-	-	-	-	-	-	-	-	-	-	-	++	! 10P5	
54		-	-	-	-	-	-	-	-	+	-	-	-	10P5	

Check 54 on liquid 10P5. - 12N7(1)

. . . 54 is TL Meth.

→	M	MT	ML	MTL	TL
	-	-	-	++	-

Hydrolysate C.

166a

200 (2+100) i/o 72 hour culture. Centrifuge cell slurry,
put in 10 ml 6 N HCl, seal tube + keep in boiling H₂O for 24 h.

lost during hydrolysis.

Try again.

7/31 - 4/

167a.

Mutants by ultra-violet irradiation.

10A 31. Add 50 ml / 125 ml flask coli C-M ε 58 (Tatum's biotinless coli) and grow on shaker, slowest speed, at room temperature.

① 9A1 - 1 ml sample to 50 ml coli C-M.

②. Irradiate in quartz tube, 11 cm from tube, 15 ~~sec.~~ min.
Add 1 ml into 50 ml coli C-M. Grow 1, 2 or shakers.

No appreciable growth in 24 hours. Dosage too high.

Try 5 mins.

1 colony at 1:50 dil.

P2 finally came up.

A2. Use 167A1 + irradiate as above, 5 min. Do in trip.
11A2.

Estimate (a) before irradiation.

Dil $\frac{1}{500 \cdot 500 \cdot 50}$ + plate into YBG. 1a.

b. after irradiation.

1:1	50^0	710.
1:50	50^1	9
1:2500	50^2	
1:125000	50^3	
1:6250000	50^4	

c. In (b) prepare last dilution in saline also. Irradiate + compare sal. + H₂O after 48 h. Do in trip. 1c.

d. Test colonies from b (1:1) and use for 58%
studies:

2a + 2c 10P2. O O
3a + c 9³⁰P3.

~~#t.~~ 15 minute irradiation A1.

Before irradiation, plate counts not made

after 1:50 -- 1.

After C-110 1 ml. 2P~~4~~ dilute $10^{-2} \times 10^6$ + reconstituted T₁,
plates 1-5 for mutants
(T₁O) + dr. mut. i)

Colonies first apparent 7P 4. 10A5 Layer YBG. (Agar too soft).

1 2 Plates so soft as
7P H. 3 to break & needles.4
5 Pads colonies before synerg. k, > 8/radiations -
YBG test on T₁O - all green - constant.

5 minute irradiation: A2 See 172

Control plate counts: *1a - (last dilution)

1:12,500,000. *1c - last dilution.

After irradiation, 1b : 1 ml. 710 { nothing, different incubate &
1:50 7 } shall alternate.c) Viability of control in sal, H₂O. - Apparently very low in this dilution

Incubate last dilution 10P2 2a 0 0

Flask of 1a, 1c example. 10P2 2c 0 0

Plate in YBG P3 3a 0 0

P3 3c 0 0

F.Sal
trinity
Study.

- I 10A1. halo in diam. = 1 cm fringe around H.C.
- Notting over 10^{-3} dilutions By 8P, there was a very faint response to H at 8P, > 3 cm diameter.
- II Supplement 10A1. By 2P, a distinct turbidity was visible, ~~over~~ over H.C. & a faint one over histidine. By 8P this was very distinct & sl. less impactive over histidine. Both ca 2.5 cm diameter..
- * 3) Supplement other portions of both plates, as above, 8³⁰P. (after prolonged incubation)
- 10^{30} P - 1B - turbidity under H.C.
— 1 better.
- 12 hour incubation probably optimal inoculum size is more or less optional & maybe reduced for frequently occurring types.
- 3) Add H.C 9P4. - No response clear?

brubations; hence at ca 35° unless stated.

1689.

Data - autoradiography

8P31 - Plate heavily (ca 10^6) into T(0) 2% agar E-8 (histidine)

I Add, as cooling deoys of HC + histidine (10%, .1% resp.)
+ dil. 1:1000 resp.

II Incubate 1/4 h. first.

III Cover & incubate to P4. Then ~~lodge~~ add ~~mannitol~~ HC to determine survival.

P2 - # 48 adopted colonies.

P4 ✓ Try more cold agar.

IV 4/2-3. Try as above \pm 3, 4, 6% agar.

No difference to speak of.
 \therefore 2% is best

V 4/3 - Use of indicator - plate E-8 as above \pm 10% /cc
Methyl Red (also 20% / 50%). Medium is alkaline to
the indicator.

See 161.

169

4/2/46.

I

Plate 161 - The area is 20% T(0) agar plate. Add suppl. HC + Biotin to surface 10P

10A - Turbidity increased over HC.

~~Extremely~~ clearer ~~under~~ under Biotin. (Cooffel 1v/ml)

5P. do. The biotin area is definitely less turbid than the rest of the plate. The plate is fairly dense but somewhat clearer under HC.

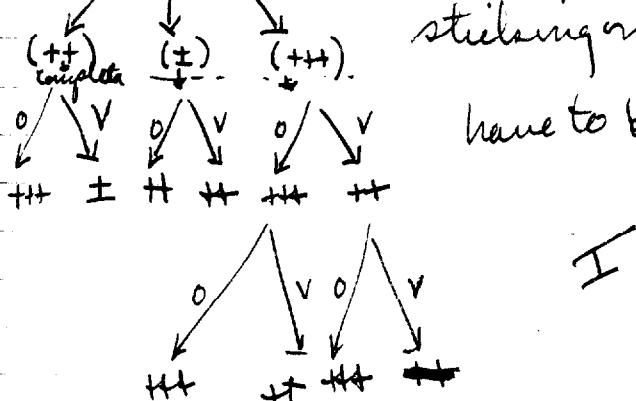
9P4. Differentials essentially disappeared. ∴ 156-9 is not an adaptation, but perhaps a slow grower & perhaps inhibited by biotin. Check L15! (At least adaptation is not genetic or "mutational")

See 173.

Study of 156-9.

1. Isolated by Method C. from γ -rayed L15.
2. Responds rapidly to HC, but grows on min.
3. Is slower on vitamins, or on biotin, but eventually grows up.
4. No "adapted" colonies seen on auxanography on 1st attempt (163.)
5. Exp. 161. Grown on HC, Vits and O. (Exp. 160). Then transferred to complete slants, growth tested on O + V. To all appearances, that grown on minimal was most sensitive to biotin. Apparently an exposure to HC or to V limits response to V, but not enough to complete. Also, growth on minimal reinforces susceptibility. This is not supported

Isolate by the final results on the HC line!!! The fundamental nature of the phenomenon is not clear. It should be more striking on plate than otherwise. Also, all these results have to be checked.



I

modulus 10^6



130

Data

Mutant Reversions

110a.

Drop ca. 1 ml of inoculum (ca. 10^6) into T/0, as indicated and incubate 48 hours; look for adapted colonies.
Rate as 110a..

Mustard.

E-3-Histidine Sep 168

58. (Bestim).
 E-6 Methionine. } Probably excess carry over: plates turbid.

Mustard ridges - Transiently rock mutant also.



Check for sister segment: 58-165a

Sex.

4/3/46.

171

SP3 - Cross streaks on minimal plate:

E-6 (methionine), 58 (biotin) + TL.

P5

no growth.

9:30 P5 - In minimal liquid, the following:

reg.

1	679-680	T-L
2	"	"
3	"	"
4	58-278	B-6
5	"	
6	Both	
7		
8		

No growth by N9.
any by P11

10P10 (1^{st} drop $\times 10^8/\text{cc}$)

N9. Repeatability is heavier, fresher inocula. in T10).

1	TL	\pm	\pm
2	TL	\pm	\pm
11	TL + B ϕ_1	\pm +	++
12	TL + B ϕ_2	+	++
13	TL + B ϕ_1	+	++
14	B ϕ_2	\pm	++
21	B ϕ_1	\pm	++
22	B ϕ_1	\pm	++
31.	In complete	TL + B ϕ_1	-
32	=	TL + B ϕ_2	-
33	(+) test (in T10)	B ϕ_1 + B ϕ_2	+ $\overset{B}{\pm}$ ++
		$T(0)^{1030}_{P9-P10} - P10^{930P10}$	+ +
		P11.	$T(0)^{930}_{P10} - P11.$

See 176. + +++

Note

Try
plating

out

from C-M.

- 2P9 Cross streaks on T(0) agar
41. B $\phi_1 \times$ TL something happened in the complete cultures
42. B $\phi_2 \times$ TL. nothing by P14. between the 1st + 2nd tests in mean.

19427 A.C.
After 20 days + 9 days
of growth at 30°C

172

Plate #	Total.	Mutants	Mutants	# s.y.	1.	Rate.
		1230 A.7	1130 A.7		1230 A.7	
1	218	3 1-3	1 4		1 5	
2	213	4 6-9	5 10-14		2 15-18	
3	220	2 11-18	3 19-21		1 22	
4	194	5 22-7	3 28-30		2 31-2	
lost	5 209	2 33-6	2 35-6		0	
6	160	1 31	0		0	
37	2 1214	17	14		6	
Y(58-)	T(b)	HC	Vits.	Auxanogram.		
1	+					
2	+					
3	+					
4	58-Y11	—✓		HC; D?; ?;		
5	+					
6	+					
7	+					
8	+					
9	+					
Proline	10 Y12	+ ±		A-15 C?		
11	58-Y12	- x+		nutrid plate Sl. 146. HC		
12	Y13	+ -				
13	58-Y13	- x+		nutrid plate		
14	58-Y13	+ -		B?		
15	Y14	+				
16		+				
17		+				
18		+				
20		+				
21						
22						
23						
24						
25						
26						

Ultra-Violet Radiation: See 167A.

Irradiate 58 coli. 5 min at 11 cm in quartz tube on shaker.

Baculum before radiation / ml. ~~($\times 10^{-8}$)~~. (8×10^{-8}).

$$\begin{array}{l} i. 1\text{g.}(455) = 5.7 \times 10^9 \\ ii. 1\text{g.} 97. = 1.23 \times 10^9 \\ iii. 1\text{g.} 300 = 3.75 \times 10^9 \end{array}$$

$$\frac{107}{3}$$

$$= 3.6 \times 10^9$$

$$\begin{array}{l} 1\text{c. } (249) = 3.12 \times 10^9 \\ 1\text{c. } (129) = 1.64 \times 10^9 \\ 1\text{c. } (252) = 3.1 \times 10^9 \end{array}$$

$$\frac{7.8}{3}$$

$$2.6$$

$$3 \times 10^9 = \text{mean.}$$

$$\text{Survivors} = \frac{710}{7100000} = \frac{7 \times 10^2}{3 \times 10^9} = 2 \times 10^{-7}$$

[Since there were ca. 5.0/ml after 15 min., there must be heterogeneity in u-v susceptibility.]

Shake 48 hours + diff. 12.5×10^6 . (9P4). Plate II in T(3).

2:30 P6. Layer.

12:30 A7. Pick mutants (ca. 1%). See data.

[See 183]

All mutants this time were picked 21 hours after layering.
Score in this group is 5/12.

3 proline.

5 mutants in 1200 cells.

comp. exp.
5 / 6000.

sp ur.	found	exp.
	5	1.7
	5.	8

$\frac{10}{7200}$ - Exp. are too small.

$$\frac{(8-5)^2}{5} = 1.8 = \chi^2 \text{ too small for sign.}$$

4/5/46

156-9 vs. L15.

173

Biotin

Inoz. 3P ♀ abund.

(1) 9A10.
(2) 9P10

	O	O	V.+	Biot.	value	
L15	+++	-	+++ ✓	-	+++ + + +	+++ ✓
156-9.	++	✓	++ ✓	-	++ ++	++
K12	+++ ✓	+	++	-	+++ -	++

Biotin is then not the only factor & there is some inhibition by a vitamin
of 156-9.

Add 1 drop 1N HCl / 10cc
for preservation?

Also consider:

Eff. pH vidop (for prep.
bryozoan preservation).

Concentration & amt. of substrate.
(Reactor might be stronger after pre-mixing bath).

4/9/46.

Cyanography-

174

Optimal inoculum size. Plate into T(B) varying samples
of an inoculum contg. ca 10^8 /cc. of 58-278 (B-φ)

incubate 12 hours & supplement w HC + w Ø. (B4 = Phenylalanine)

Inoc. 530P.9 Suppl. 1030 9A10.

Diam. of lawn HC.

1 ml 1 1.8 ++ 2.3 +++

.1 ml 2 2.5 ++ 3cm ++

.02 ml 3 2cm ++ 3cm +

.002 ml 4 ? ± 2cm +

.000004 ml 5 #2 + 4cm +

7 ↑

distinctness

The method can be used at any
inoculum size, but is most acc
& best suited. For very adaptable
strains, it may be important.

1:25,0000

OPTIMAL AGAR DEPTH. 4/10.

Inoc. 1ml undil culture
into varying agar depths. Inoc. ~~1030~~ incubate to 1130P9.
Supplement

A - φ - at f = 0. B: φ at 1230P11.

A (IIA) 730P B. 230P

1 5ml

2 10ml

3 15ml

4 30ml

nothing *partially*
nothing *nothing* *bit*
nothing *nothing* *no growth*

It makes very little
difference what
depth agar is used
10-15 ml is quite OK.

TIME OF INCUBATION.

Inoc. 58-278 into T(B) agar 9P11

t 9A12 7P12

Suppl. ①

10P

0

±

optimal < 12h.

② 12M

+

+

③ ~~10A12~~

-

-

quality of
growth

④ 10A12

#1

-

⑤

-

-

⑥

-

-

⑦

-

-

sample is 6000.
ca. 5 mutants

Compare 175.

hydrolysate A 1:10.

* Contains γ Neurospor + thermophil!

4/9 - 10... 156.

Spontaneous mutants in 58.

175

530P9	broz	t.t.	ϵ coli	cay	58.	shake at RT.
9P10-dil	1: 12,500,000	+ plate out by method II in T(biotin).				
Inoculate at 35° to 11 P11		Incubate complete.				Plates covered!
Korut 1130A/T2	6 P13.					
*1						
*2						
*3						
4	1000	1				
5	"	2-5				
6	"	6				
7	"	7	8-9			
8	"	10				
9	"		11,12			
*10						
12 h. O 304.						
Add .1 ml of "1/10" in lieu of biotin.						
11.						
Picks to cay 830P14 n.v. satisfactory						
None of the 1st series would have qualified except by streaking						
retests. 1(B). <u>U.v.</u> more than catalogued.						
1	+					
2	+					
3	-					
4	+					
5	+					
6	+					
7	-					
8	-					
9	-					
10	no growth in CM					
11	-					
12	n.v. int M.					

Jex.

921

4/12/46

Broc. C-M E:	& shaleae			A12 - P13			
1. TL							
2. BΦ ₁							
3. TL+BΦ ₁							
12 M13.	T(0)	P16.	P17	P17: add	A18	P19.	
Test 1) 4	-	-	-	loopful of	-	-	
2) 5	-	-	-	complete	-	-	
3) 6	±	+++		coli medium			
1+2) 7	±	+++		to tube			
				4, 5 and 9			
3) v.s.m. 8	-	-	-	13, 15 + 17			
1+2) 9	-	-	#-		-	-	hilly?
See 171. Plate 31. into T(0) + cover. M13. (TL × BΦ ₁ , v.c-m).							
1: 25000	10	No colonies P16	Covered Φ A16 + B.	Not in frame up	P17	A18	P18
1: 12,500,000	11						
12) 3 into CM, 3 shaking at 30° 1030 A13							
small mor. 13) Test: o. - - # +							
large mor. 14) ++ +++							
P16. Test 6 m 15	-	+++ #					
6 large 16	-	+++					
7 m 17	-	- #	-				
7 large 18	-	-	-				

Steranography.

Preservatives.

I Plate K-12 in T/0). Add drops of HCl-diluted.

Centrifuge 4 hrs 10N 4 hrs inhibition

2 1:10

1 hrs inhib.

4 1:100

No inhibition

try 1 drop HCl/
10cc water, etc.

6 1:1000

No inhib.

8 1:10⁴

No inhib.

II. Benzene.

1. Centrifuge - pure Benzene Clear zone

2. 10 - Benzene-water. OK

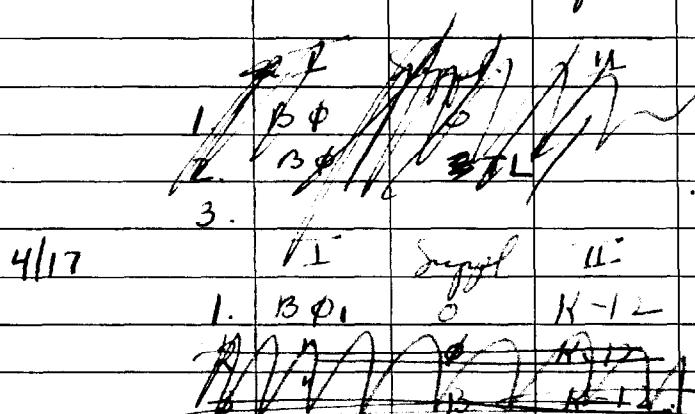
3. 5 - Alcohol 95%. OK

4. ~~10~~ Chloroform OK.

Syntrophism

179

Pour plates \approx ca 10^6 organisms arrived + suggest. where
hard, streak II. on surface.



See N-363 for prep on symbiosis with N: 33757.
~~ME~~ E 181.

A19. About each streak is a clear zone, mixed \in a range
of greater density, fading off to a small turbidity.

On complete P19 - No differential zone found. H-12 somewhat
nihilated.

Salt resistance mutants.

180

after Stevens & Tanner.

4/13/70. 581

1. Plate ~~H~~, ca 10^3 , into complete plates +
1. CuSO_4 . ~~-~~ g 10 mg
2 MgCl_2 .1 mg

A19. Nothing came up. use less.

A *Penicillium* sp. contaminant
did grow on D!

Syntrophus

181.

58-161 + 58-273
14 \$

10 ml T(B) in den. tubes. Biotin .01% Suppl. + monolite as indicated (in v/10ml): 1030 P19. incubate at ~~28°~~ 28°

Dens. readings (galer. uncorrected).

#	Moi	Sup: \$	M	930P20.	1P21	330P22	9P22	9P23	Tube #
1	M	10	10	-	+	80	80	+1.5	
2	\$	10	10	-	-	93 ²	95 ²	-	!
3	M	0	0	-	-	93 ²	93 ²	-	
4	\$	0	0	-	-	94 ²	93 ²	-	
5	K-12	0	0	++	+++	58 ²	57 ²	+++	
11	\$+M.	1	0	-	-	94	93 ²	-	
12	"		1	-	±	90	83	+++	
13	"		3	-	±	68	61 ²	+++	
14	"		10	-	-	83	75 ²	+++	
21	"	3	0	-	-	94 ¹	94 ²	-	
22	"		1	-	-	93 ¹	93 ¹	±	
23	"		3	-	-	75 ¹	63 ¹	+++	
24	"		10	-	+	66	67	+++	
31	"	10	0	-	±	88	84	+++	
32	"		1	-	-	93	92 ¹	±	
33	"		3	-	+	84	13 ³	+++	
34	"		10	-	±	81 ³	80 ²	+1.5	
41	"	0	0	-	-	92 ¹	92 ²	-	
42	"		1	-	-	92	91 ¹	±	
43	"		3	-	-	93	93	-	
44	"		10.	-	-	95 ¹	90	-	

75% = $\frac{1}{2}$ max.

73² 73¹

45. Plate \$+M\$ into plates & for methionine; top for phenylalanine (plate to see whether this is a good separation). ~~rel. inadequate~~

#22 to 38°. This does not spread things up.

#13

Dil 1:50,50,500 = ~~1:2,500,000~~ = 1:2,500,000. + moi. into

A. T(B) $\frac{1}{2} 0$

B. T(B+M) 40 21.5%

C. T(B+G) 146

4/19/46
26 old!

As before:

		<u>Supplements</u>		FPA.
5	brcz	Ø	Tyr.	
51	K-12	0	0	10r
52		0	0	100r
53		10r	0	100r
54		100r	0	100r
55		0	10r	100r
56		0	100r	100r
57		10r	10r	100r
58	58-278	1r	0	0
59		10r	0	0
60		100r	0	0
61		10r	100r	0
62		10r	0	10r
63		10r	0	50r
64		10r	0	100r
65		0	10	100r
66		100r	0	10r
67		100r	0	50r
68		100r	0	100r
69		10r	10r	100r
70		0	0	50r
				100r
71	58-4899	0	0	100r
72	58-5030	0	0	100r
73		0	10	0
74.		100	10	0

Many aspects of this experiment all coextensive with the investigation of the aromatic a.a. mutants + are to be postponed until this is carried through by E.L.T., et.al.

4/19. Utilization of FPA:

		430P20	3P22	9P22	P23
81	58-278	10	-	96	-
82		100	-	97	-
83	58-4899	10	-	96 ²	-
84		100	-	97	-
85	58-5030	10	±?	95	75 ³ ±
86		100	-	67	65 ¹ ±
					+++ (green tint)

† test as minimal: they grow.

Mutant detection:Viability

(24 hours)

Pour 58-278, dil. to 1:12,500,000 into T(b) plates + cover as in mutant detection. 830 P19. Cover & complete at time t₁. Colony diameter recorded at t₂. Incubate at 38°.

* have h¹ 430P20 h² 830P 10A21 4P21 8P21 12M21 Count.

1	4P19	1	19	5	+++ (residue) variation.	
2	"	1		4	← do	362
3	1130	3	17		+++	352
4	"	3		6	← do	—
5	930A20	12	7	< #1.	+++	379
6	"	12		3	← do.	407 (41?)
7	830P20	23		—	++	363
8	"	23		—	+++	—
9	10A21	36		—	++	9.380. ← too many bottom colonies
10	8P21	48.			++	349
11	—	—			++	353
12	+ 1mg S	—				
13	—	—	6			

Bottan colonies troublesome. 19A22 353
1 hour is barely too long for period II. Last runs 4.9.

Incubum from 48 hour stock culture is complete. Incubate 30°.

21	9P20	8	+	++	++	++	0	131	- 9
22	9P20.	9	+	++	++	++	0	139	-
23	1P21			±	+	++	16	138	- 2
24	1P21.			±	+	++	16	151	+ 11
25	8P20	23		—	+	++	23	154.	+ 14
26	8P20	26		—	+	++	23	133	- 7
27	930A22						36	139	- 1
28							123	- 87	
29	3P23	7:15P.	⑤	colonies quite distinct inc. indicated (3-5 h.)	++	++	64	156.	+ 16
30	1130A24.	28°	130°	—	7 mm. colonies	7 mm. colonies	145	+ 5	
31	"	30°			⑧	⑧	135	- 5	
32	" (2x)	38°	colonies min. visible	—	3 1/2 da.	86.	340. (2x)		
33	C.M.-P.C.	+	+++				0	146	+ 16
34	Plate count	+	+++				0	143	+ 13
35	"	+	+++				0	138	+ 18
								A.	
								Count. $\Sigma A^2 =$	
								m = 140.	1097.
								$\sigma = \sqrt{m} - \bar{x}$	8.85
								$\sigma_{\text{calc.}} = \sqrt{\frac{m}{n}}$	14.

11A21. 8P21 12M21 10A22 P23

Viability of 58-278 at 38° is excellent for 48 hours.

* 7 small colonies noted 10P21 puncture.

Rich colonies 11A30. see 194.

* 15 units = 1 num.